MOLECULAR AND CELLULAR ENDOCRINOLOGY, Vol. 73, January 1990, pages R13-R18) in view of HIMMLER et al. ("Functional Testing of Human Dopamine D_1 and D_5 Receptors Expressed in Stable cAMP-Responsive Luciferase Reporter Cell Lines", JOURNAL OF RECEPTOR RESEARCH, Vol. 13, No. 1 /04, January 1993, pages 79-84).

It is not believed to be accurate to state that one of ordinary skill in the art at the time the invention was made, would have been motivated to substitute the direct measurement of cAMP taught by LUDGATE …, for the luciferase enzymatic determination taught by HIMMLER et al., with a reasonable expectation of success. It must be remembered that the inventors of the subject matter of the present claims were not in possession of the present discovery when trying to solve the problem of how to improve the assay disclosed in LUDGATE et al. in order to provide a practically workable assay for use in detecting TSH-R auto-antibodies or TSH. The guidelines for examination specifically caution against applying the benefit of hindsight in order selectively to highlight certain documents in the prior art and to use them, as a mosaic, to arrive at the claimed subject matter. At the date the invention was made, in September 1997, the inventors of the presently-claimed subject matter were beginning from the point they had arrived at, which is described in LUDGATE et al. The question they were faced with was how to overcome the numerous disadvantages of this assay, not least the length of time it takes to perform, especially in view

of having to detect the generated cAMP by radio immuno-assay, and also the fact that the method requires tissue culture facilities to be available.

It would not be unreasonable to assume that, if one had no inventive skill, when trying to find an improvement for the existing assay, one would first look towards assays for related substrates i.e. substrates related to TSH-R auto-antibodies or TSH. It may not be unreasonable to suppose that an appropriate place to start looking would be amongst G-protein coupled receptors (GPCR). However, there are currently more than 1,000 GPCR, of which more than 300 are of human origin. GPCR couple to a range of signaling pathways, including adenylate cyclase (both stimulatory and inhibitory), inositol phophate, and calcilum signaling and ion channels. The applicant has, in fact, searched in computer databases (such as BIDS) for papers relating to GPCR that are also concerned with adenylate cyclase or cAMP. This search gives rise to many hits regarding the biology of the receptors, but nothing relevant to novel detection methods; HIMMLER et al. is not included amongst these hits. Furthermore, BIDS searches using cAMP detection, cAMP assay, cAMP responsive reporter, cAMP or signal transduction as key words also do not give rise to any useful results. In particular, amongst these searches, either HIMMLER et al. does not appear or there are so many hits that it is not feasible to review them all. It is therefore difficult to see how the solution finally arrived at

(of using the particular construct as defined in the claims) would have been obvious in the light of such prior art.

Even were the HIMMLER et al. citation to have appeared in any of these searches or even were the inventors otherwise aware of the HIMMLER et al. paper, it is difficult to see how they would have been led to believe that its disclosure provided the solution to the problem of a TSH-related assay. It is highly significant that the HIMMLER et al. paper focuses on certain dopamine receptors with a view to testing the response of putative dopamine receptor drugs. The dopamine receptor family comprises five subtypes, several of which have further subvariants. Although D_1 and D_5 couple to adenylate cyclase (stimulatory), the other three sub-types inhibit adenylate cyclase or are coupled to ion channels. Hence, the dopamine receptor family is by no means an obvious one with which to draw an analogy for the TSH receptor. HIMMLER et al. itself points out (page 91) that other dopamine systems based on CAT do not lend themselves to such an assay. It is difficult to see how, when a system based on the same receptor type cannot be assumed to work in a particular assay, it could be assumed that a similar system would work on a completely different kind of receptor.

Furthermore, none of this takes into account the fact that there are significant differences in the construct disclosed by HIMMLER et al. and that used in the present invention (see Example 1 and revised claims). The construct selected was that

described by KRISHNA et al. ("Repression of the Human Glycoprotein Hormone α-Subunit Gene by Glucocorticoids: Evidence for Receptor Interactions with Limiting Transcriptional Activators", MOL. ENDOCRINOLOGY, Vol. 5, No. 1, January 1991, pages 100-110) in the reference specified on page 4, line 30 of the application as filed (copy supplied with our Information Disclosure Statement of March 30, 2000). This construct, as illustrated in Figure 1 of KRISHNA et al., is clearly described as having a tandem repeat of the CRE. This is quite different from the structure of HIMMLER et al.'s construct, which has multiple CREs. From HIMMLER et al. can be seen that the HIMMLER et al. construct comprises three CREs. Still further, the HIMMLER et al. CREs are taken from heterologous sources and synthetically combined to produce the construct, whereas those of KRISHNA et al. are naturally present in the glycoprotein hormone alpha sub-unit. It is understood that these two differences are highly significant in their effect on the cell line in the assay for which it is intended to be used. There is already published evidence of the fact that the artificial, three CRE-containing construct of HIMMLER et al. is significantly inferior in use than the tandem-repeated, naturally-derived construct of the cell line of the present invention.

The applicant specifically refers to the study reported, after the date of the present invention, by WATSON et al. in "A new chemiluminescent assay for the rapid detection of

thyroid stimulating antibodies in Graves' disease", CLIN. ENDOCRINOLOGY, Vol. 49, November 1998, pages 577-581 (copy supplied with our Information Disclosure Statement of March 30, 2000) in which the HIMMLER et al. construct was used in a chemi-luminescent assay for the detection of thyroid stimulating antibodies (TSAB) in Graves' disease. WATSON et al. find 60% of all (treated and not) Graves patients positive using the HIMMLER et al. construct. This compares with 73% (of 100) in comparable experiments using the KRISHNA et al. construct as in the present invention, as quoted in EVANS et al. J Clin Endocrinol Metab (1999) (copy attached). Furthermore, as in the WATSON et al. tests, they found only 80% of 14 untreated Graves patients positive, which compares with 97% of 40 untreated Graves' patients in a comparable assay using the cell line of the present invention (data not yet published). In addition, using the cell line according to the present invention, there is obtained a maximal 25-fold increase in light output in response to bovine TSH, compared with only a 9-fold increase reported in the WATSON et al. paper.

Accordingly, these data show that the presently-claimed cell line is significantly superior in its ability to be used in an assay for the detection and quantification of TSAB and the like when compared with the WATSON et al. cell line, which difference depends not only on the difference in the cAMP responsive luciferase construct used but also on the numbers of

TSH receptors expressed by each cell type. It is noted that each paper (WATSON et al. and EVANS et al.) states that the cell line selected was the best of those obtained by the transfection.

Thus the choice of the KHRISNA et al. construct in the development of the cell line of the present invention would not have been obvious in the light of these comparable experiments. The KHRISNA et al. construct was first reported in 1989 and was therefore available to both HIMMLER et al. and WATSON et al. at the date they developed their cell line/assay. In particular, it was available to WATSON et al. when they decided to attempt a TSH-related assay in 1998. Nevertheless, WATSON et al. chose the HIMMLER et al. construct, whereas, a year earlier, the present inventors selected the KHRISNA et al. construct, with unexpectedly effective results. The WATSON et al. research demonstrates that the use of the KHRISNA et al. construct was both non-obvious, and the comparative data show that the choice of the present inventor gave unexpectedly good assay results.

As these distinctions are clearly brought out in the claims as now amended, it is believed that they are all patentable, and reconsideration and allowance are respectfully requested.

Attached hereto is a marked-up version of the changes made to the claims. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

YOUNG & THOMPSON

By _____

Robert J. Patch
Attorney for Applicant
Registration No. 17,355
745 South 23rd Street
Arlington, VA 22202
Telephone: 521-2297

April 22, 2002

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claim 36 has been amended as follows:

36. (amended) A clone expressing human TSH-R stably transfected with a reporter construct comprising cDNA of both

(i) a reactant[, such as an enzyme,] capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein

and

(ii) a promoter containing cAMP response elements (CREs), comprising a promoter sequence or synthetic oligonucleotide which contains the CRE consensus sequence, TGACGTCA,

whereby levels of the reactant vary with induced endogenous cAMP levels; and

wherein the promoter sequence or synthetic oligonucleotide is that for the glycoprotein hormone alpha subunit that contains a tandem repeat of the CRE consensus sequence, TGACGTCA.

Claim 40 has been amended as follows:

40. (amended) cDNA or mRNA expressing human TSH-R stably transfected with a reporter construct comprising cDNA of both

(i) a reactant[, such as an enzyme,] capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein

and

(ii) a promoter containing cAMP response elements (CREs), comprising a promoter sequence or synthetic oligonucleotide which contains the CRE consensus sequence, TGACGTCA,

whereby levels of the reactant vary with induced endogenous cAMP levels; and

wherein the promoter sequence or synthetic oligonucleotide is that for the glycoprotein hormone alpha subunit that contains a tandem repeat of the CRE consensus sequence, TGACGTCA.